CONCENTRATION-DEPENDENT VASCULAR EFFECTS OF DIVALENT MANGANESE

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OBJECTIVES**:** Divalent manganese is a cofactor for soluble guanylyl cyclase (sGC), the enzyme producing cyclic GMP in vascular smooth muscle cells that causes relaxation. Manganese competes with magnesium to activate sGC. These divalent cations can bias the activity of the enzyme to produce cyclic nucleotides other than cyclic GMP, in particular cyclic AMP and cyclic IMP. Cyclic IMP, preferably produced in the presence of magnesium, can cause contraction in precontracted arteries of the pig. The objective of the present study was to identify the mechanisms by which manganese, compared to magnesium [used in standard physiological solutions at millimolar concentrations for functional studies], affects endothelium-dependent and -independent relaxations by influencing intracellular levels of cyclic nucleotides.

METHODS**:** *Ex vivo* experiments were designed using isolated rat aortae of Sprague-Dawley rats of 12-14 weeks of age and porcine coronary arteries (collected at the local abattoir). The arteries were cut into rings and used for measurement of vascular reactivity in conventional organ chambers using pharmacological inhibitors of endothelial nitric oxide (NO) synthase (LNAME) and of cyclooxygenases (indomethacin). In some rings, the endothelium was removed through insertion of a wooden toothpick. After obtaining an optimal resting tension, the rings were treated with millimolar concentrations of manganese or magnesium for fortyfive minutes and precontracted with phenylephrine (rat aortae) or U46619 (porcine coronary arteries). Subsequently, concentration-dependent responses were obtained with acetylcholine (rat arteries), bradykinin (porcine coronary arteries) and the NO-donor SNP. In parallel, *in vitro* experiments were conducted using cultured porcine coronary artery smooth muscle cells (PCASMCs). The cells were exposed to millimolar concentrations of manganese or magnesium in the presence or absence of a sGC-activator (YC-1). Intracellular cyclic GMP levels were measured using a commercially available ELISA kit. RESULTS**:** *In vivo*, millimolar ranges of manganese significantly decreased endothelium-dependent relaxations to acetylcholine in rat aortae and to bradykinin in porcine coronary arteries compared to physiological levels of magnesium. This effect was abolished by endothelium-removal. In addition, L-NAME partially reversed the effect of manganese, while indomethacin did not. Manganese also decreased, but to a smaller extent, endotheliumindependent relaxations to SNP in both porcine coronary arteries and rat aortae. In parallel, the in vitro study showed that manganese alone or in combination with YC-1 blocks the production of cyclic GMP.

CONCLUSIONS**:** Taken into conjunction, these experiments show that manganese at millimolar levels decreases endothelium-dependent and –independent relaxations compared to physiological levels of magnesium. This is likely explained through a decrease in cyclic GMP production. The effect of manganese is endothelium-dependent. In the case of endothelium-dependent relaxations (to acetylcholine or bradykinin), the effect of manganese is mediated by endothelial NO synthase, but not by cyclooxygenases. These findings indicate that in the presence of manganese NO does not act as a vasodilator and must lead to production of cyclic nucleotides other than cyclic GMP to cause contraction, similar to what has been reported under hypoxic conditions.